Exposure to Ginger Root Oil Enhances Mating Success of Irradiated, Mass-Reared Males of Mediterranean Fruit Fly (Diptera: Tephritidae)

TODD E. SHELLY[1] AND DONALD O. MCINNIS[2]

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ABSTRACT Previous research revealed that exposure to ginger root oil, *Zingiber officinale* Roscoe, containing the known male attractant (α-copaene) increased the mating success of male Mediterranean fruit flies, *Ceratitis capitata* (Wiedemann), from a newly established laboratory colony. The goal of the current study was to determine whether exposure to ginger root oil likewise enhanced the mating competitiveness of irradiated *C. capitata* males from a 5-yr-old mass-reared strain. Mating tests were conducted in field cages containing guava trees (*Psidium guajava* L.) to monitor the mating frequency of irradiated, mass-reared and wild males competing for wild females. In the absence of chemical exposure, wild males outcompeted the mass-reared males and obtained 74% of all matings. However, following exposure to ginger root oil (20 μl for 6 h), the mating frequencies were reversed. Whether exposed as mature (3-d-old) or immature (1-d-old) adults, mass-reared males achieved $\approx$75% of all matings in tests conducted 2 or 4 d following exposure, respectively. Irradiated, mass-reared males prevented from contacting the oil directly (i.e., exposed to the odor only for 6 h) still exhibited a mating advantage over wild males. In an additional study, signaling levels and female arrivals were compared between males exposed to ginger root oil and nonexposed males, but no significant differences were detected. The implications of these findings for the sterile insect technique are discussed.

KEY WORDS *Ceratitis capitata*, sterile insect technique, ginger root oil

THE STERILE INSECT technique is an environmentally benign approach for suppressing or eradicating insect pests and is widely used in control efforts against tephritid fruit fly pests, particularly the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Hendrichs et al. 1995). The technique involves mass production of males of the target species and release of sterilized males (via irradiation) into the environment. Matings between sterile males and wild females yield infertile eggs, which reduces the reproductive potential of the wild population. Because the success of the sterile insect technique depends on the ability of mass-reared, sterile males to copulate with wild females, it is essential that the mass-rearing protocol itself does not produce males with diminished mating competitiveness (Calkins 1984). This consideration is especially important for species, such as *C. capitata*, in which females display a high degree of mate discrimination, based apparently on male courtship performance (Whittier et al. 1992, 1994).

Unfortunately, the mass-rearing procedures inherent to the sterile insect technique often lead to a reduction in the mating competitiveness and viability of released *C. capitata* males, particularly in long-established strains (Shelly et al. 1994, Lance et al. 2000). The deterioration of *C. capitata* strains results from a combination of factors, including genetic drift with its concomitant loss of genetic variability and intense artificial selection imposed in the laboratory (Leppla and Ozaki 1991). Because of these problems, sterile males typically have low mating success relative to wild males (Rossler 1975, Shelly et al. 1994, McInnis et al. 1996, Lance et al. 2000). Aside from changing strains frequently, there is currently no effective way to avoid this decrease in mating competitiveness, and for the recently developed genetic sexing strains (e.g., temperature sensitive lethal), strain changes require considerable time and effort for the development of new and genetically stable translocations (Franz et al. 1996).

Thus, a persistent and important challenge for the sterile insect technique is the development of simple and inexpensive means to enhance the mating performance of released, sterile *C. capitata* males in the wild. Based on prior research in Hawaii (Shelly 1999, 2001), one potentially productive approach involves the prerelease exposure of males to particular attractants that have been shown to enhance their mating success in field cage tests. Experiments with wild-like males (i.e., from a newly established laboratory strain) demonstrated a strong effect of ginger root oil (*Zingiber officinale* Roscoe, containing the known male attractant α-copaene; Flath et al. 1994a,b; Nishida et al. 2000) on male mating success. Mature, wild males exposed to the odor of ginger root oil obtained 81% of all matings in competitiveness tests with exposed conducted 2 d after exposure (Shelly 2001). Additional

[1] USDA–APHIS, P.O. Box 1040, Waimanalo, HI 96795; and Hawaiian Evolutionary Biology Program, University of Hawaii, Honolulu, HI 96822.

[2] USDA–ARS, PBARC, 2727 Woodlawn Drive, Honolulu, HI 96822.

tests showed that (1) exposure to immature males (1 d old) conferred a pronounced mating advantage in tests conducted 8 d after exposure, and (2) exposure to the odor of ginger root oil alone was sufficient to improve mating performance.

The goal of the current study was expand upon these previous results and to determine whether exposure to ginger root oil likewise enhanced the mating competitiveness of irradiated *C. capitata* males from a 5-yr-old mass-reared strain. We also compared male calling (signaling) activity and mate attraction between exposed and unexposed mass-reared males. The implications of the present findings for the sterile insect technique are discussed.

Materials and Methods

Mating Behavior of *C. capitata*. The Mediterranean fruit fly exhibits a lek mating system (Prokopy and Hendrichs 1979, Hendrichs and Hendrichs 1990, Shelly et al. 1994). Males defend individual leaves on host and nonhost trees as mating territories and, while perching, emit a pheromone attractive to females by everting their rectal epithelium and expanding their abdominal pleura (a behavior hereafter termed calling or signaling; Arita and Kaneshiro 1986). Following female arrival to the territory, males perform courtship involving visual, acoustic, and olfactory cues and then mount the female (Arita and Kaneshiro 1989). Copulation generally lasts 90–180 min (Seo et al. 1990). Females may reject males by simply departing before mounting or dropping from the leaf after mounting. Females display a high level of mate selectivity, and <10% of courtships observed in a laboratory study resulted in copulation (Whittier et al. 1994).

Flies. Wild flies were reared from infested coffee berries (*Coffea arabica L.*) collected on Kauai. Fruits were held over vermiculite at 23–25°C, and larval development proceeded in situ. Pupae were sifted from the vermiculite 7–9 d after fruit collection, and adults were separated by sex within 2 d of eclosion, well before reaching sexual maturity at 7–10 d of age (T.E.S. and D.O.M., unpublished data). Mass-reared males were from the Maui-Med strain produced by the USDA–APHIS Hawaii Fruit Fly Rearing Facility, Waimanalo, HI, since 1996. Pupae of this mass-reared strain were exposed in air to 15 krad of gamma irradiation from a ^{137}Cs source 2 d before eclosion and then delivered to the laboratory. Males were collected within 12 h of eclosion (males of this strain attain sexual maturity at 2–3 d of age; T.E.S. and D.O. M., unpublished data). Both wild and irradiated, mass-reared adults were held in plastic buckets covered with nylon screening (volume 5 liters; 100–125 flies per bucket). Adults were provided with a sugar-protein mixture (3:1 wt:wt) and water ad libitum, held at 20–24°C and 65–85% RH, and received both natural and artificial light with a photoperiod of 12:12 (L:D) h.

Male Mating Success. Mating tests were conducted at the Agricultural Experiment Station of the University of Hawaii, Waimanalo, Oahu. Groups of 100 irradiated, mass-reared males, 100 wild males (10–15 d old) and 100 wild females (10–16 d old) were released between 0800 and 0830 hours in field-cages (2.5 m in height, 3.0 m in diameter) that contained a single rooted guava tree. For a given trial, we marked only males from one group (i.e., mass-reared or wild) and alternated the identity of the marked group between successive trials. Males were marked 1 d before testing by cooling them for several minutes and placing a dot of enamel paint on the thorax. This procedure had no obvious adverse effects, and males resumed normal activities within minutes of handling. The cages were monitored continuously for 4 h, mating pairs were collected in vials, and the males identified. Individuals of both sexes were virgins when tested, and new flies were used in every trial.

We ran six experiments. With the exception of experiment 6, the wild males were not exposed to ginger root oil. To gather baseline data on their mating competitiveness, the irradiated, mass-reared males used in experiment 1 (5–7 d old) were not exposed to ginger root oil. In experiment 2, mass-reared males were exposed to ginger root oil when 3 d old (i.e., mature) and tested 2 d later. In experiment 3, mass-reared males were exposed when 1 d old (i.e., immature) and tested 4 d later.

Both experiments 2 and 3 used the following "standard" procedure for exposing mass-reared males. We applied 20 μl of ginger root oil (this dose was used in all subsequent experiments as well) to a small disk (4 mm in diameter) of filter paper with a microcapillary pipette. The oil, obtained from Citrus and Allied Essences, Lake Success, NY, contains the male attractant α-copaene in low concentration (0.4% by volume, T. W. Phillips, personal communication) along with other sesquiterpenes whose effect on *C. capitata*, either independently or in combination with α-copaene, are unknown. The disk was placed on the bottom of a transparent, plastic drinking cup (400-m volume), 25 males were immediately placed in the cup using an aspirator, and the cup was covered with nylon screening. Exposure started at 0700 hours and continued until 1300 hours. The behavior of males was not monitored systematically during exposure periods, but in frequent checks, males were never observed touching the paper disk, a finding consistent with Nishida et al. (2000). Rather, the chemical acted as an arrestant, and males were generally quiescent. Following exposure, treated males were removed from the exposure cups, placed in holding buckets, and moved to an adjacent room. The exposure procedure was conducted in a room isolated from any flies to prevent the inadvertent exposure of control males.

In experiments 4 and 5, mass-reared males were exposed using slightly modified procedures (as before, wild males were not exposed in these experiments). In experiment 4, mature, mass-reared males (5 d old) were exposed to ginger root oil (20 μl) for 1 h only (0600–0700 hours) just before their use in a mating test. In experiment 5, we attempted to confirm whether the changes observed in mating competitiveness (described below) following exposure were effected by the aroma of the ginger root oil alone and did

not require direct contact with the oil-containing disks. We placed the disks in small containers (covered with nylon mesh screening) that were introduced into the plastic cups. Thus, males were exposed to the chemical's aroma but were unable to contact the source directly. Males were exposed for 6 h (0700–1300 hours) when 3 d old and tested 2 d later.

In the final experiment, we exposed both mature, mass-reared (3 d old) and wild (10–12 d old) males to ginger root following the standard procedure and tested them 2 d later. Because wild males may seek and gather fragrances in the wild (under *Discussion*), experiments 2–5, which used chemically naive wild males, may have unduly favored the exposed mass-reared males in mating competition. By comparing the results of this final experiment with experiment 1 (where males of both strains were chemically naive), we could examine the relative importance of chemical exposure versus interstrain differences in affecting the mating success of mass-reared and wild males.

It should be noted that, in previous work (Shelly 2001), exposure of wild-like pupae to ginger root oil had no apparent effect on the mating frequency of subsequently eclosed males. Consequently, no experiments involving pupal exposure were included in the current study.

Male Calling and Female Attraction. In an additional experiment, we examined whether ginger root oil affected the calling activity of irradiated, mass-reared males and long-range attraction of females. Data were collected in a large field tent (15 m in length by 6 m in width by 2.5 m in height) that included 17 rooted guava trees in two rows running the length of the tent. Groups of five mass-reared males of a given type (exposed or nonexposed) were placed in transparent, plastic cups (400-m volume with both ends covered with wire screening) and suspended in four trees, with two trees having exposed males exclusively and two having nonexposed males exclusively. Males receiving the treatment were exposed to the oil when 3 d old following the standard procedure and tested 2 d later. Three cups, containing a total of 15 males of a given type, were placed on each of the trees at 0730–0800 hours, at ≈1.7 m above ground in shaded sites in the outer canopy. Cups on a given tree were placed in the same area of the canopy, so that distances between them did not exceed 0.3 m. The test trees were located at the corners of the tent.

Ten minutes after male placement, 100 females (9–14 d old) were released from the center of the tent. After an additional 10 min, the number of calling males and females at each cup were recorded at 10-min intervals for 120 min ($n = 13$ observations per tree per replicate). Females that were perching on or within 15 cm of a cup were collected with an aspirator and placed in a holding bucket. This procedure appeared to have negligible impact on male calling activity or the behavior of other females. Tests were conducted on 7 d under sunny or partly sunny conditions with temperatures ranging from 22 to 26.5°C. The same release point and the same four trees were used on each of the seven test days. For each tree, the type of male present (exposed or non-exposed) was alternated between successive trials. Because some females invariably remained in the tent after a replicate (i.e., did not respond to the calling males), females were marked with enamel paint on the previous day (following the above-mentioned procedure) to allow identification of those females released on a given test day. If sighted, females released on previous test days were collected but not included in the counts. Owing to a shortage of wild flies, the females used in this experiment were from a laboratory colony started with 200–300 adults reared from coffee collected on Oahu. This stock was maintained following the methods outlined in Shelly (2001), and when used in this experiment, the females were four generations removed from the wild.

Statistical Analyses. Mating frequency was described using the mean number of matings (±SE) per replicate, though statistical comparisons between control and treated males were made using the nonparametric Mann-Whitney test (test statistic T). Because this test does not explicitly test for deviation from random mating (i.e., 50% of the matings by each male type), a binomial test (using the normal approximation with test statistic Z) was performed using data pooled over all cages. Proportions of matings obtained by wild males in experiments 1 and 6 were compared using the G test with Yates correction for continuity. In the female attraction experiment, data from the three cups in the same tree were combined for each observation, and these values were averaged over all observations (13 per replicate) to obtain a single measure of male calling and female arrivals for a given replicate. These summary values were used to compare exposed and nonexposed males in a Mann-Whitney test.

Results

Male Mating Success. In the absence of any chemical exposure, wild males outcompeted irradiated, mass-reared males in the mating trials. In experiment 1, wild males obtained an average of 20.8 matings (±2.9) per replicate compared with only 7.3 (±1.3) for mass-reared males ($T = 125.0$, $n_1 = n_2 = 9$, $P < 0.001$). Over all replicates, wild males accounted for 74% (187/253) of the matings ($Z = 8.6$, $P < 0.001$).

In contrast, when irradiated, mass-reared males alone were exposed to ginger root, mating frequencies of the two strains were reversed, and mass-reared males had a pronounced mating advantage (Table 1). Mass-reared males obtained significantly more matings than wild males in all experiments in which males were exposed as adults, i.e., experiments 2–5 (Table 1). Binomial tests for random mating also revealed significantly biased mating frequencies favoring treated males in these experiments. Mass-reared males accounted for the following proportion of total matings ($P < 0.001$ in all cases): experiment 2, 75% (217/291, $Z = 9.6$); experiment 3, 72% (223/311, $Z = 8.5$); experiment 4, 82% (246/299, $Z = 14.5$), and experiment 5, 79% (247/312, $Z = 12.6$).

Table 1. Effect of ginger root oil on the mating success of mass-reared *C. capitata* males under different conditions of exposure

Experiment[a]	Conditions of exposure			Post-exposure interval, d	Matings per replicate		T^a
	Male age, d	Duration, h	Contact possible?		Mass-reared	Wild	
2	3	6	Yes	2	27.1 (2.5)	9.2 (2.0)	98.0*
3	1	6	Yes	4	27.9 (2.5)	11.0 (1.8)	99.0*
4	5	1	Yes	0	30.8 (3.3)	6.6 (1.1)	100.0*
5	3	6	No	2	30.9 (3.2)	8.1 (1.3)	100.0*

* Wild males were not exposed to ginger root oil in any of these experiments. Numbers of matings are means; standard errors are given in parentheses.

[a] Eight replicates were conducted for experiments 2–5.

[b] $P < 0.001$.

In experiment 6 in which both mass-reared and wild males were exposed to ginger root oil, wild males (mean = 32.5 ± 2.2) achieved, on average, a significantly greater number of matings than mass-reared males (mean = 21.5 ± 1.7; $T = 91.0$, $n_1 = n_2 = 8$, $P < 0.05$). Over all replicates, wild males accounted for 60% (260/432) of the total matings ($Z = 4.3$, $P < 0.001$). Both male types achieved, on average, more matings per replicate in this experiment than in experiment 1 (neither male type exposed) ($T = 98.5$ and 105.0 for wild and mass-reared males, respectively; $n_1 = 9$, $n_2 = 8$; $P < 0.05$ in both cases). Although wild males had a mating advantage in both of these experiments, exposure of both male types to ginger root oil lessened this advantage: wild males achieved a larger proportion of matings in experiment 1 (74%) than experiment 6 (60%) ($G = 13.0$, df = 1, $P < 0.001$).

Male Calling and Female Attraction. On average, 5.7 (±0.6) exposed males were calling within an aggregation (tree) per observation compared with 5.3 (±0.4) nonexposed males ($T = 214.0$, $n_1 = n_2 = 14$, $P > 0.05$). The average number of females collected per observation was also similar between aggregations of exposed (mean = 0.9 ± 0.1) and nonexposed (mean = 0.6 ± 0.1) males ($T = 235.0$, $n_1 = n_2 = 14$, $P > 0.05$). These data suggest that female arrivals calculated per calling male were similar between groups of exposed (mean = 0.16 ± .02) and nonexposed (mean = 0.13 ± .01) males ($T = 220.0$, $n_1 = n_2 = 14$, $P > 0.05$).

Discussion

Exposure to ginger root oil dramatically increased the mating success of irradiated, mass-reared males of the Mediterranean fruit fly. Without exposure, mass-reared males obtained ≈25% of all matings, but with exposure they obtained 72–82% of all matings. Levels of postexposure success were similar across the different exposure regimes, and strong positive effects were observed following exposure (1) of immature and mature males, (2) for short (1 h) and long (6 h) periods, (3) immediately or 2–4 d before the mating test, and (4) with or without possible contact with the oil-bearing source. The results of experiment 6 showed that, when both male types were exposed to ginger root oil, wild males had higher mating success than mass-reared males, although this advantage was significantly lower than that observed when neither male type was exposed to ginger root oil. This finding suggests that, even if wild males are successful in locating natural sources of α-copaene (the presumed active ingredient of ginger root oil), exposing mass-reared males to ginger root oil still enhances their mating competitiveness (over nonexposed males), and prerelease exposure is thus a potentially valuable tool in the sterile insect technique.

The factor(s) underlying the postexposure increase in mating performance remains unknown. Based on the mate attraction experiment, it does not appear that exposure to ginger root oil enhanced male signaling activity or long-range attractiveness. This result contrasts with results obtained for wild-like flies (12 generations removed from the wild), where exposed males called more frequently and had more female visits than nonexposed males (Shelly 2001). Because neither signaling activity nor long-range attractiveness appeared important for mass-reared males, future work on laboratory strains should perhaps focus on comparing the performance of individual courtship behaviors as well as the composition of close-range pheromonal signals between exposed and nonexposed males.

Regardless of the underlying mechanism, the chemically induced increase in mating success suggests several potential benefits to the sterile insect technique. Most importantly, prerelease exposure might effectively counteract any decline in competitive ability resulting from the mass-rearing procedure. In effect, this would "rescue" poorly performing strains by obviating the need to replace them, thus increasing the operational lifespan of laboratory strains. In this regard, it is noteworthy that exposure to ginger root oil appears to have no adverse effects on flight ability or longevity of irradiated, mass-reared males (T.E.S., unpublished data). By increasing the quality of mass-reared males, chemical exposure also might reduce the production demands of mass-rearing facilities (because fewer males are required for suppression or eradication) or alternatively increase the availability of mass-reared flies among different control programs (because fewer males are required per program). In addition, the release of more competitive males may accelerate suppression or eradication efforts and thereby reduce the duration of control programs and associated costs. Finally, as described above, the methods used for prerelease exposure are simple and rel-

atively inexpensive (the current cost of ginger root oil is $30/kg), thus making the procedure readily available to control programs.

Additional research is required to determine more precisely the beneficial impact of prerelease exposure on the sterile insect technique. Of perhaps highest priority, tests should be conducted on a larger spatial scale, where the effectiveness of exposed and nonexposed sterile males in depressing populations is measured either in large field cages or the wild. Concomitantly, methods need to be developed for delivering ginger root oil effectively to large batches of flies, e.g., 36,000–40,000 adults held in prerelease boxes. To assess the general utility of prerelease exposure, tests should be conducted with different strains of mass-reared males from different rearing facilities throughout the world. Preliminary data (T.E.S. and D.O.M., unpublished data) collected in Guatemala are encouraging in this regard: exposure to ginger root oil appears to double the mating frequency of males from the Vienna-7 genetic sexing strain (currently used in the Moscamed Program) in competition against wild males in field cages. Studies also are required to examine possible negative effects of exposure on male longevity. For example, in studying the effects of adult nutrition on the performance of sterile males of *C. capitata*, Kaspi and Yuval (2000) reported that the addition of protein to the diet increased mating success but reduced survival. Based on casual observations, no such tradeoff occurs following exposure to ginger root oil, but rigorous tests have yet been conducted. Finally, prerelease exposure to male attractants should be examined for other tephritid species. For example, Shelly and Dewire (1994) and Tan and Nishida (1996) have demonstrated that males of several *Bactrocera* spp. fed the attractant methyl eugenol have a pronounced mating advantage over unfed males. Thus, where the sterile insect technique is required to achieve eradication of these species, prerelease feeding of methyl eugenol may increase the likelihood of success and also hasten the process.

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